

CC (RAPLRI) with Met at position 1 attached to (His)6 tag and Met24Leu.
 CC Carboxy terminal end of recombinant RAPLRI has a covalently bound ligand
 CC binding moiety, which can be a LL2 antibody directed against CD22 on
 CC cancerous B cells or human chorionic gonadotropin (hCG) effective
 CC against Kaposi's sarcoma cells. Recombinant ribonucleases can be
 CC expressed in bacteria without an N-terminal methionine due to the
 CC presence of a signal peptide that is cleaved by bacteria. The soluble
 CC expression of ribonuclease allows the proteins to be fused in-frame with
 CC ligand binding moieties to form cytotoxic fusion proteins. They can be
 CC used for treatment of cancer and autoimmune diseases.

XX Sequence 105 AA;

Query Match 99.5%; Score 575; DB 20; Length 105;
 Best Local Similarity 99.0%; Pred. No. 4.1e-62;
 Matches 103; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

OY 1 QDWLTQKKHLTNTRPVDCNNIMSTNLFHCKDKNTFTYSRPEPKAICKGIASKNVLTTS 60
 Db 2 QDWLTQKKHLTNTRPVDCNNIMSTNLFHCKDKNTFTYSRPEPKAICKGIASKNVLTTS 61
 OY 61 SEFYLSDCNWTSPCKYKLLKSKTNTFCVTCENQAPVHFVGVGHC 104
 Db 62 SEFYLSDCNWTSPCKYKLLKSKTNTFCVTCENQAPVHFVGVGHC 105

RESULT 6
 AAY28870
 ID AAY28870 standard; Protein; 104 AA.

XX AAY28870;

DT 25-JAN-2000 (first entry)

XX Recombinant RAPLRI Gln1Ser amino acid sequence.

XX Recombinant Rana pipiens ribonuclease; RAPLRI Gln1Ser; covalently bound;
 KW LL2 antibody; ligand binding moiety; CD22; cancerous B cell; frog;
 KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
 KW recombinant ribonuclease; cytotoxic fusion protein; cancer; RNase;
 KW autoimmune disease.

OS Rana pipiens.
 OS Synthetic.

XX Key Location/Qualifiers

FT Misc-difference 1 /note="Wild type Gln replaced with Ser"

PN W09950398-A2.

PD 07-OCT-1999.

PF 26-MAR-1999; 99WO-US06641.

PR 27-MAR-1998; 98US-0079751.

XX (USSH) US DEPT HEALTH & HUMAN SERVICES.

PI Newton DL, Rybak SM;

DR WPI: 1999-610847/52.

DR N-PSDB: AA208128.

XX New recombinant ribonucleases, used for killing target cells, e.g. for
 PT treating cancers, viral infections or autoimmune diseases -

PS Claim 34; Page 60; 71pp; English.

XX The present sequence is a recombinant Rana pipiens ribonuclease (RAPLRI)
 CC protein with Gln1Ser. Carboxy terminal end of recombinant RAPLRI has a
 CC covalently bound ligand binding moiety, which can be a LL2 antibody
 CC directed against CD22 on cancerous B cells or human chorionic

CC gonadotropin (hCG) effective against Kaposi's sarcoma cells. Recombinant
 CC ribonucleases can be expressed in bacteria without an N-terminal
 CC methionine due to the presence of a signal peptide that is cleaved by
 CC bacteria. The soluble expression of ribonuclease allows the proteins to
 CC be fused in-frame with ligand binding moieties to form cytotoxic fusion
 CC proteins. They can be used for treatment of cancer and autoimmune
 CC diseases.

XX Sequence 104 AA;

Query Match 99.1%; Score 573; DB 20; Length 104;
 Best Local Similarity 100.0%; Pred. No. 7.1e-62;
 Matches 103; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

OY 2 DWLTFQKKHLTNTRPVDCNNIMSTNLFHCKDKNTFTYSRPEPKAICKGIASKNVLTTS 61
 Db 2 DWLTFQKKHLTNTRPVDCNNIMSTNLFHCKDKNTFTYSRPEPKAICKGIASKNVLTTS 61
 OY 62 EFLYLSDCNWTSPCKYKLLKSKTNTFCVTCENQAPVHFVGVGHC 104
 Db 62 EFLYLSDCNWTSPCKYKLLKSKTNTFCVTCENQAPVHFVGVGHC 104

RESULT 7
 AAY28871
 ID AAY28871 standard; Protein; 105 AA.

XX AAY28871;

DT 25-JAN-2000 (first entry)

XX Recombinant Met(-1) RAPLRI Gln1Ser amino acid sequence.

XX Recombinant Met(-1) Rana pipiens ribonuclease Gln1Ser; RAPLRI; CD22;
 KW covalently bound; LL2 antibody; ligand binding moiety; cancerous B cell;
 KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
 KW recombinant ribonuclease; cytotoxic fusion protein; cancer; frog;
 KW autoimmune disease; RNase.

OS Rana pipiens.
 OS Synthetic.

XX Key Location/Qualifiers

FT Misc-difference 1 /note="Met not found in wild type RAPLRI"

FT Misc-difference 2 /note="Wild type Gln replaced with Ser"

PN W09950398-A2.

PD 07-OCT-1999.

PF 26-MAR-1999; 99WO-US06641.

PR 27-MAR-1998; 98US-0079751.

XX (USSH) US DEPT HEALTH & HUMAN SERVICES.

PI Newton DL, Rybak SM;

DR WPI: 1999-610847/52.

DR N-PSDB: AA208129.

XX New recombinant ribonucleases, used for killing target cells, e.g. for
 PT treating cancers, viral infections or autoimmune diseases -

PS Claim 34; Page 61; 71pp; English.

XX The present sequence is a recombinant Rana pipiens ribonuclease (RAPLRI)
 CC protein with Met at position 1 and Gln2Ser. Carboxy terminal end of
 CC recombinant RAPLRI has a covalently bound ligand binding moiety, which
 CC can be a LL2 antibody directed against CD22 on cancerous B cells or human
 CC chorionic gonadotropin (hCG) effective against Kaposi's sarcoma cells.


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XX XX Frog; ribonuclease; ranpirinase; RNase.
XX XX Rana pipiens.
OS XX
XX XX Key Location/Qualifiers
XX XX Modified-site 1
XX XX /note="this Gln is autocyclised to pyroglutamic acid"
XX XX US6175003-B1.
XX XX 16-JAN-2001.
XX XX
XX XX 10-SEP-1999; 99US-0394268.
XX XX
XX XX 10-SEP-1999; 99US-0394268.
XX XX
XX XX (ALFA-) ALFACELL CORP.
XX XX
XX XX Saxena SK.
XX XX
XX XX WPI: 2001-167808/17.
XX XX
XX XX New nucleic acids encoding a ribonuclease (RNase); useful for the
XX XX precise targeting of RNase to a predetermined cell receptor.
XX XX
XX XX Claim 1: Columns 5-6; 7pp; English.
XX XX
XX XX The present sequence represents a frog ribonuclease protein (ranpirinase)
XX XX (RNase). The specification describes a synthetic ribonuclease protein,
XX XX in which the addition of cysteine in the ribonuclease facilitates the
XX XX chemical linking of a targeting molecule by the single reactive
XX XX sulfhydryl group. The specification also describes a method for the
XX XX production of ranpirinase using DNA technology instead of processing
XX XX biological material. The re-engineering of the protein molecule allows
XX XX easier attachment to a targeting molecule thereby making it possible for
XX XX the ribonuclease to be delivered to a particular cell receptor where it
XX XX might be most effective.
XX XX
XX XX Sequence 104 AA:
SO
Query Match 96.2%; Score 556; DB 22; Length 104;
Best Local Similarity 96.2%; Pred. No. 8.3e-60;
Matches 100; Conservative 2; Mismatches 2; Indels 0; Gaps 0;
OY 1 QDWLTFQKKHLNTRDVCNINIMSTNLFHCKDKNFTYSRPEPKAIGIASKNVLT 60
DB 1 QDWLTFQKKHLNTRDVCNINIMSTNLFHCKDKNFTYSRPEPKAIGIASKNVLT 60
OY 61 SEFYLSDCNVTSPCKYKRLKSTNFTFCVCENQAPVHFGVGC 104
DB 61 SEFYLSDCNVTSPCKYKRLKSTNFTFCVCENQAPVHFGVGC 104
RESULT 11
AAW35126
ID AAW35126 standard; Protein; 379 AA.
XX
XX AAW35126;
XX
XX 20-APR-1998 (first entry)
XX
XX R. pipiens recombinant RNase ronc fusion protein 2.
XX
XX RNase A; ribonuclease; cytotoxic; onconase; nonc; immunofusion;
XX tumour cell growth; frog.
XX
XX Rana pipiens.
XX Synthetic.
XX
XX W09731116-A2.
XX
XX 28-AUG-1997.

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XX XX 19-FEB-1997; 97WO-US02588.
XX XX
XX XX 21-FEB-1996; 96US-0011800.
XX XX
XX XX (USSH ) US DEPT HEALTH & HUMAN SERVICES.
XX XX
XX XX Boque L, Newton DL, Rybak SM, Wlodawer A;
XX XX WPI: 1997-435168/40.
XX XX N-PSDB: AAT94964.
XX XX
XX XX Ribonuclease molecules based on native Onconase - used for killing
XX XX cells, particularly tumour cells
XX XX
XX XX Disclosure: Page 68; 90pp; English.
XX XX
XX XX Sequences AAW35125 to AAW35135 represent recombinant fusion proteins
XX XX (ronc) which are modifications of the RNase Onconase (RM) (nonc). Such
XX XX novel ribonuclease molecules are highly cytotoxic and can be used alone
XX XX or to form chemical conjugates or to target recombinant immunofusions.
XX XX They are used particularly for decreasing tumour cell growth. They can
XX XX also be used for cell separation in vitro by selectively killing unwanted
XX XX types of cells, e.g. in bone marrow prior to transplantation into a
XX XX patient undergoing marrow ablation by radiation, or for killing leukaemia
XX XX cells or T-cells that would cause graft versus host disease. The toxins
XX XX can also be used to selectively kill unwanted cells in culture. The new
XX XX ribonucleases have increased cytotoxic activity compared to nonc and
XX XX also lower immunogenicity in humans.
XX XX
XX XX Sequence 379 AA:
SO
Query Match 96.2%; Score 556; DB 18; Length 379;
Best Local Similarity 96.2%; Pred. No. 4.4e-59;
Matches 100; Conservative 2; Mismatches 2; Indels 0; Gaps 0;
OY 1 QDWLTFQKKHLNTRDVCNINIMSTNLFHCKDKNFTYSRPEPKAIGIASKNVLT 60
DB 26 QDWLTFQKKHLNTRDVCNINIMSTNLFHCKDKNFTYSRPEPKAIGIASKNVLT 85
OY 61 SEFYLSDCNVTSPCKYKRLKSTNFTFCVCENQAPVHFGVGC 104
DB 86 SEFYLSDCNVTSPCKYKRLKSTNFTFCVCENQAPVHFGVGC 129
RESULT 12
AAR12344
ID AAR12344 standard; protein; 104 AA.
XX
XX AAR12344;
XX
XX 08-AUG-1991 (first entry)
XX
XX Protein with activity against cancer cells.
XX
XX Frog eggs; Tamoxifen; Stelazine; cancer.
XX
XX Rana pipiens.
XX
XX W09107435-A.
XX
XX 30-MAY-1991.
XX
XX 26-OCT-1990; 90WO-US06185.
XX
XX 18-MAY-1990; 90US-0526314.
XX
XX 13-NOV-1989; 89US-0436141.
XX
XX (ALFA-) ALFACELL CORP.
XX
XX Ardelt WJ, Mikulski SM;
XX
XX WPI: 1991-178059/24.

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XX New protein from fertilised eggs of Rana pipiens - active against
PT cancer cells, esp. in combination with Tamoxifen or Stelazine
PT (trifluoro-per-azine).
PS
PS Claim 7; Fig 2; 33pp; English.
XX
XX The protein is derived from fertilised frog eggs. It has an iso-
CC electric point of 9.5 - 10.5, a blocked N-terminal gp. and is free
CC of carbohydrates. It is active against certain cancer cells. The
CC combination of the protein and (2-1-p-dimethylaminoethoxyphenyl)-1,
CC 2-diethyl-1-butene citrate salt (Tamoxifen) is much more bio-
CC active than the separate entities against human pancreatic ASPC-1
CC adenocarcinoma, and the combination of protein and (10-(3-(4-methyl
CC piperazin-1-yl)-propyl)-2-trifluoromethylphenothiazine (Stelazine)
CC is much more reactive than the separate entities against human lung
CC A-549 carcinoma. Activity has also been shown against human sub-
CC maxillary epidermoid carcinoma A-253 cells, human ovarian adeno-
CC carcinoma NIH-OVCAR-3 cells, human Leukaemic HL-60 cells, human
CC COLO 320 DM cells, human LOX melanoma and human lung squamous car-
CC cinoma HT-520 cells.
XX
XX Sequence 104 AA:
SQ
Query Match 95.7%; Score 553; DB 12; Length 104;
Best Local Similarity 95.2%; Pred. No. 1.9e-59;
Matches 99; Conservative 3; Mismatches 2; Indels 0; Gaps 0;
QY 1 QDWLTFQKHULTNRDVCNNIMSTNLFHCKDKMTFTYSRPEPKAICKGITASKNVLT 60
DB 1 EDWLTFFQKHITNRDVCNNIMSTNLFHCKDKMTFTYSRPEPKAICKGITASKNVLT 60
QY 61 SEFLSDCNVTSRCKYKRLKSTNTFCVTCENQAPVHFVGSHC 104
DB 61 SEFLSDCNVTSRCKYKRLKSTNTFCVTCENQAPVHFVGSHC 104
RESULT 13
AAR47303
ID AAR47303 standard; protein: 104 AA.
XX
AC AAR47303;
XX
DT 09-SEP-1994 (first entry)
XX
DE ONCONASE (pharmaceutical protein).
XX
KW Onconase; pharmaceutical; protein; adenocarcinoma; treatment;
KW cisplatin; melphalan; adriamycin; ovarian cancer; ovary.
XX
OS Synthetic.
XX
PN WO9403197-A.
XX
PD 17-FEB-1994.
XX
PF 02-JUL-1993; 93MO-US06357.
XX
PR 30-JUL-1992; 92US-0921180.
XX
PA (ALFA-) ALFACELL CORP.
XX
PI Ardelit WJ, Mikulski SM;
XX
DR WPI; 1994-065396/08.
XX
PT Pharmaceutical contg. Cisplatin, Melphalan or Adriamycin - active
PT in-vitro against OVCAR-3 human ovarian adenocarcinoma cells
XX
PS Claim 7; Page 13; 18pp; English.
CC This pharmaceutical protein (ONCONASE) is used in the production of
a bioactive pharmaceutical composition also comprising one of

CC Cisplatin (cis-diamminedichloroplatinum), Melphalan, (4-[bis-(2-
CC chloroethyl)amino]-L-phenylamine) or Adriamycin (Doxorubicin HCl).
CC The composition has bioactivity in vitro against OVCAR-3 human
CC ovarian adenocarcinoma cells.
XX
XX Sequence 104 AA;
SQ
Query Match 95.7%; Score 553; DB 15; Length 104;
Best Local Similarity 95.2%; Pred. No. 1.9e-59;
Matches 99; Conservative 3; Mismatches 2; Indels 0; Gaps 0;
QY 1 QDWLTFQKHULTNRDVCNNIMSTNLFHCKDKMTFTYSRPEPKAICKGITASKNVLT 60
DB 1 EDWLTFFQKHITNRDVCNNIMSTNLFHCKDKMTFTYSRPEPKAICKGITASKNVLT 60
QY 61 SEFLSDCNVTSRCKYKRLKSTNTFCVTCENQAPVHFVGSHC 104
DB 61 SEFLSDCNVTSRCKYKRLKSTNTFCVTCENQAPVHFVGSHC 104
RESULT 14
AAM00736
ID AAM00736 standard; protein: 104 AA.
XX
AC AAM00736;
XX
DT 22-MAY-1997 (first entry)
XX
DE Protein derived from frogs eggs.
XX
KW Rana pipiens; ovarian adenocarcinoma NIH-OVCAR03 cell; frog; egg;
KW submaxillary epidermoid carcinoma A-253 cell; tumour; human;
KW leukaemic HL-60 cell; COLO 320 DM cell; colon adenocarcinoma;
KW LOX melanoma; lung squamous carcinoma HT-520 cell.
XX
OS Rana pipiens.
XX
PN US559212-A.
XX
PD 24-SEP-1996.
XX
PE 06-APR-1988; 88US-0178118.
XX
PR 03-FEB-1992; 92US-0814332.
XX
PR 06-APR-1988; 88US-0178118.
XX
PR 13-NOV-1989; 89US-0436141.
XX
PR 01-AUG-1994; 94US-0283970.
XX
PA (ALFA-) ALFACELL CORP.
XX
PI Ardelit WJ;
XX
DR WPI; 1996-442459/44.
XX
PT New isolated Rana pipiens frog protein - useful for the treatment of
PT tumours.
XX
PS Claim 1; Column 8; 7pp; English.
XX
XX This sequence represents a protein which was prepared by homogenisation
CC of Rana pipiens frogs eggs. This protein is used for treating tumours
CC in humans. Especially this protein was active against human
CC submaxillary epidermoid carcinoma A-253 cells, human ovarian
CC adenocarcinoma NIH-OVCAR03 cells, human leukaemic HL-60 cells, human
CC COLO 320 DM cells originally isolated from colon adenocarcinoma, human
CC LOX melanoma and human lung squamous carcinoma HT-520 cells.
XX
XX Sequence 104 AA;

Query Match 95.7%; Score 553; DB 17; Length 104;
Best Local Similarity 95.2%; Pred. No. 1.9e-59;
Matches 99; Conservative 3; Mismatches 2; Indels 0; Gaps 0;

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QY      1 QDWLTFQKKHLTNTRDVDCNNIMSTNLFHCKDKNTFIYSRPEPYKAICKGIITASKNVLT 60
      :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db      1 EDWLTFQKKHLTNTRDVDCNNIMSTNLFHCKDKNTFIYSRPEPYKAICKGIITASKNVLT 60
QY      61 SEFYLSDCNVTSRPCKYKLRKSTNTEFCVTCENQAPVHFVGVGHC 104
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db      61 SEFYLSDCNVTSRPCKYKLRKSTNTEFCVTCENQAPVHFVGVGSC 104

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RESULT 15

AAW06543
ID AAW06543 standard; protein: 104 AA.

AAW06543:

22-AUG-1997 (first entry)

DE Antitumour protein from Rana pipiens oocytes.

KW Tumour; chemotherapy; radiotherapy; frog.

OS Rana pipiens.

PN W09639428-A1.

PD 12-DEC-1996.

PF 03-JUN-1996; 96MO-US08304.

PR 06-JUN-1995; 95US-0467955.

PA (ALFA-) ALFACELL CORP.

PI Argelt WJ:

DR WPI: 1997-043063/04.

PT Antitumour proteins from Rana pipiens oocyte(s) - have fewer
disadvantages than chemotherapy, surgery and radiotherapy

PS Claim 7; Page 27; 45pp; English.

XX The present sequence is a specifically claimed example of an
CC antitumour protein from the generic protein in AAW18224, with the
CC molecular weight 12000. This is one of two preferred proteins (the
CC other in AAW06544) that have been isolated from Rana pipiens oocytes.
CC Both proteins have a blocked amino terminal group and are essentially
CC free of carbohydrates. The proteins are used to treat tumours. Use of
CC the peptides has fewer disadvantages than chemotherapy, radiotherapy
CC and surgery in the treatment of tumours.

SQ Sequence 104 AA:

Query Match 95.7%; Score 553; DB 18; Length 104;
Best Local Similarity 95.2%; Pred. No. 1.9e-59;
Matches 99; Conservative 3; Mismatches 2; Indels 0; Gaps 0;

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QY      1 QDWLTFQKKHLTNTRDVDCNNIMSTNLFHCKDKNTFIYSRPEPYKAICKGIITASKNVLT 60
      :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db      1 EDWLTFQKKHLTNTRDVDCNNIMSTNLFHCKDKNTFIYSRPEPYKAICKGIITASKNVLT 60
QY      61 SEFYLSDCNVTSRPCKYKLRKSTNTEFCVTCENQAPVHFVGVGHC 104
      |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db      61 SEFYLSDCNVTSRPCKYKLRKSTNTEFCVTCENQAPVHFVGVGSC 104

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